

*Modification of sucrose synthase gene expression in plant  
tissue and uses therefor*

Related Applications

- 5 This application is a continuation-in-part of U.S. Provisional Application Serial No. 60/251,852, filed December 8, 2000, the contents of which are incorporated herein by reference.

Technical Field

- 10 The present invention relates to modifying targeted gene expression in plants to obtain a desired effect in the plant.

Background Art

- 15 Sucrose synthase (SuSy) is a key enzyme in the breakdown of sucrose in all plant sink tissue, including grain and fruit and has been extensively studied in many plants. Only relatively recently, however, have this protein and gene been characterized from cotton. The full length (2625 bp) of cotton sucrose synthase (SuSy) was isolated by Perez-Grau, L. and Delmer, D. in UC, Davis (accession number U73588) in May 1996. A 2030 bp of the same cDNA with  
20 595 bp missing at the 5' end was isolated by the same group in 1994 and was given to Prem Chourey in USDA/ARS for collaborative research. However, no evidence was available at that time regarding the role of this SuSy gene in cotton fiber/seed development, although it had been speculated (Amor et al., 1995) that part of the fiber localised SuSy could associate with cellulose  
25 synthase playing a role to channel carbon to this enzyme.

- Evidence has been obtained that the expression of the SuSy gene could be important not only for cellulose synthesis but also for fiber cell initiation (thus may control fuzz) and a model on how sucrose is partitioned and competed for  
30 between fiber, seed coat and embryos of the cotton seed was proposed. These work were detailed in the following two papers: Ruan et al., 1997 *Plant*

*Physiology* 115, 375-385; Ruan and Chourey, 1998, *Plant Physiology* 118, 399-406. More recently, the present inventors obtained further evidence supporting the hypothesis that SuSy plays a key role in mobilising sucrose into initiating fiber cells (Ruan et al., 2000 *AJPP* 27, 795-800).

5

The art is thus deficient in providing methods and means for altering the fiber development and properties in plants, particularly cotton, through alteration of sucrose synthase levels in cells of the plants.

- 10 These and other problems are solved as described hereinafter in the different embodiments and claims.

The present inventors have now obtained evidence regarding the role of SuSy in plant development and function through suppressing SuSy expression in

- 15 transgenic cotton.

#### Summary of the invention.

- The invention provides a method for altering fiber development or properties of a fiber-producing plant, preferably a cotton plant, particularly a Fibermax<sup>TM</sup>
- 20 variety comprising the steps of providing cells of said plants with a chimeric gene comprising the following operably linked DNA fragments: a plant expressible promoter, preferably a subterranean clover stunt virus promoter; a coding region which when transcribed yields an RNA said RNA being capable of reducing the expression of an endogenous sucrose synthase gene,
- 25 preferably an endogenous sucrose synthase gene expressed in fiber cells, preferably fiber initial cells; or capable of being translated into an active sucrose synthase protein; and a transcription termination and polyadenylation signal which functions in the plant cells.
- 30 In the embodiments where the RNA is capable of being translated into an active sucrose synthase protein, preferred coding regions comprise a

nucleotide sequence selected from the group consisting of: (a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID No 2; (b) a nucleotide sequence comprising the nucleotide sequence of SEQ ID No 1; (c) a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence a) or b); and (d) a nucleotide sequence hybridizing under stringent conditions with the nucleotide sequence (a) or (b); or a part thereof encoding an active sucrose synthase.

In the embodiments where the RNA is capable of reducing the expression of an endogenous sucrose synthase gene, preferred coding regions comprise a nucleotide sequence selected from the group consisting of: a nucleotide sequence comprising at least 19 or 25 contiguous nucleotides having at least 70 % sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID No 2 or the complement thereof; and a nucleotide sequence comprising at least 19 or 25 contiguous nucleotides having at least 70 % sequence identity from a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID No 1 or the complement thereof, particularly where the coding region comprises the nucleotide sequence of SEQ ID No 1 from the nucleotide at position 2208 to the nucleotide at position 2598 or the complement thereof. The coding region may comprise simultaneously both sense and antisense nucleotide sequences capable of forming a double stranded RNA molecule.

It is another objective of the invention to provide a method for improving fiber yield in a fiber-producing plant, comprising providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments: a plant expressible promoter; a DNA region capable of being translated into an active sucrose synthase protein; a transcription termination and polyadenylation signal which functions in said plant cells.

30

The invention also provides a method for improving fiber quality in a fiber-producing plant, comprising providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments a plant expressible promoter;

- 5 A DNA region capable of being translated into an active sucrose synthase protein a transcription termination and polyadenylation signal which functions in said plant cells.

- 10 In yet another embodiment of the invention a method is provided for increasing seed size in a fiber-producing plant, comprising providing cells of said plant with a chimeric gene comprising the following operably linked DNA fragments a seed-specific promoter; a DNA region capable of being translated into an active sucrose synthase protein; a transcription termination and polyadenylation signal which functions in said plant cells.

- 15 The invention also relates to fiber-producing plants, particularly cotton plants, comprising in their genome a chimeric DNA comprising the following operably linked DNA fragments: a plant expressible promoter; a coding region which when transcribed yields an RNA, said RNA being capable of reducing the expression of an endogenous sucrose synthase gene, preferably an
- 20 endogenous sucrose synthase gene expressed in fiber cells, preferably fiber initial cells; or capable of being translated into an active sucrose synthase protein; a transcription termination and polyadenylation signal which functions in said plant cells.

- 25 Yet another objective of the invention is to provide a fiber-producing plant comprising a chimeric DNA according to the invention wherein said RNA is capable of increasing the expression of an endogenous sucrose synthase gene, preferably an endogenous sucrose synthase gene expressed in fiber
- 30 cells, preferably fiber initial cells and said fiber cells have an increased sucrose

synthase activity compared to fiber cells of plant cells which do not comprise said chimeric DNA.

In yet another embodiment of the invention fiber-producing plants are provided comprising a chimeric DNA according to the invention, wherein said coding region comprises a nucleotide sequence selected from (a) a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID No 2; (b) a nucleotide sequence comprising the nucleotide sequence of SEQ ID No 1; (c) a nucleotide sequence having at least 70% sequence identity with the nucleotide sequence (a) or (b); and (d) a nucleotide sequence hybridizing under stringent conditions with the nucleotide sequence (a) or (b); or a part thereof encoding an active sucrose synthase.

The invention also provides a fiber-producing plant comprising the chimeric genes of the invention wherein said RNA is capable of reducing an endogenous sucrose synthase gene and said fiber cells have a reduced sucrose synthase activity compared to fiber cells of plant cells which do not comprise said chimeric DNA, particularly wherein the coding region comprises a nucleotide sequence selected from the group consisting of a nucleotide sequence comprising at least 19 or 25 contiguous nucleotides having at least 70 % sequence identity to a nucleotide sequence encoding a polypeptide comprising the amino acid sequence of SEQ ID No 2 or the complement thereof; and a nucleotide sequence comprising at least 19 or 25 contiguous nucleotides having at least 70 % sequence identity from a nucleotide sequence encoding a polypeptide comprising the nucleotide sequence of SEQ ID No 1 or the complement thereof, particularly wherein the coding region comprises the nucleotide sequence of SEQ ID No 1 from the nucleotide at position 2208 to the nucleotide at position 2598 or the complement thereof.

Also provided are seeds of a fiber-producing plant, preferably cotton plant comprising the chimeric DNA or genes according the invention as well as fibers with altered development or properties, isolated from such plants.

- 5 The invention also relates to the use of a sucrose synthase encoding nucleotide sequence for altering fiber yield or fiber quality or fiber properties.

#### Brief Description of the Drawings

- 10 Figure 1 illustrates that down regulation of SuSy in the fiber causes a decrease in fiber elongation in transgenic plants. Three developmental stages are shown: 0, 2 and 6 days after anthesis.

- 15 Figure 2 is an immunolocalization to detect SuSy protein level. Note black labelling in the wild type (WT) in developing fiber cells on surface of ovule and in ovule epidermis. Transgenic lines show reduced amount of SuSy protein (less black label) and in 295-82, no SuSy protein. This correlates with lack of fiber development.

- 20 Figure 3 shows transgenic lines where SuSy is also reduced in the embryo (upper boll and seeds). Seeds are fiberless but note the great reduction in seed size in III. Some seeds did not form at all.

- 25 Figure 4 shows that there was no effect of the transgene on vegetative growth. (WT on left and two typical transgenic lines).

- Figure 5 shows construct maps used for SuSy suppression. Note nptII is neomycin phosphotransferase marker gene for selection of transformants and S1, S7 and S3, S5 designate the promoters and terminators respectively encoded by the subclover stunt-virus (see Australian Patent No 689311).  
30 "SUSY" represents the 390 bp nucleotide sequence of a sucrose synthase cDNA (available at Genbank under the Accession number U73588) from

CTGGGAT to TGA CTT (211 bp coding region upstream of the stop codon plus 179 bp 3' UTR region; nucleotide sequence of SEQ ID 1 from the nucleotide at position 2208 to the nucleotide at position 2598).

5 Figure 6 represents Southern blot hybridization results on DNA isolated from T0 generation plants (A) and segregating plants of the T1 generation plants (B) respectively. The arrows indicate lines with fiber-less phenotypes. The numbers indicate the transgenic cotton line number to which the individual plants belong.

10

Figure 7 is a scanning electron microscopy (SEM) analysis on ovule epidermis of line 147 in Fig 2B. Many fiber cells are collapsed and shrunk, some fiber cells popped up but were much smaller and less in number (Fig 7 B) when compared to the wild type (Fig 7 A) ovule. The shrunk and collapsed  
15 phenotype was clearly demonstrated under high magnification (Fig 3 C).

Figure 8 shows the correlation between changes of SuSy activity (Fig 8A) and reduction of fiber length (Fig 8B) in 3-day-old seeds from 10 segregating individuals of three T1 generation lines (lines 147, 43 and 101), together with  
20 line 82 and wild type (WT). Figure 8C represents the regression analysis between SuSy activity and fiber length.

Figure 9 is a figure showing reduced fuzz length in SuSy suppressed transgenic line 147#2 (panel B) compared to wild type (panel B). The seeds  
25 were delinted by hand. The oval represents the chalazal end of the mature seeds. Seeds covered by fuzz of the transgenic line appear browner than the wild type seeds, because the underlying brown seed coat is covered by shorter fuzz fiber.

### Detailed Description of the Invention

In a first aspect, the present invention provides a plant having altered expression of an isoform of sucrose synthase (SuSy) resulting in an altered fiber, fruit or seed production ability.

5

Preferably, the plant is a transgenic plant having an under- or overexpression of an isoform of SuSy involved in fruit/seed production. In a preferred form, the plant retains expression of SuSy in developing vegetative tissue such that growth and development of the plant is not adversely affected.

10

When the plant undergoes underexpression of an isoform of SuSy involved in fruit/seed production, the plant has a reduced fiber or seed production. When the plant undergoes overexpression of an isoform of SuSy involved in fruit/seed production, the plant has an enhanced fiber or seed production.

15

The ability to cause underexpression of an isoform of SuSy involved in fruit/seed production can be used to develop plants that produce few or no seeds but are still able to grow and produce fruit normally. The ability to cause overexpression of an isoform of SuSy involved in fruit/seed production in a tissue specific manner can be used to develop plants which produce greater amounts of fiber or longer fiber or having altered fiber structure but are still able to grow and develop normally. The plant can be any plant where expression of an isoform of SuSy is involved in fruit/seed production. The present invention is applicable for modifying a wide range of horticultural crops such as grape, peach, pear, and apple. More preferably, the plant is a cotton plant.

25

In a preferred embodiment, plants, particularly cotton plants, are provided comprising a chimeric gene, preferably stably integrated in their genome, the chimeric gene comprising the following operably linked DNA fragments:

30

a plant expressible promoter

a coding region which when transcribed yields an RNA which is either



capable of reducing the expression of an endogenous sucrose synthase gene, preferably an endogenous sucrose synthase gene expressed in fiber initial cells; or

- capable of being translated into an active sucrose synthase protein; and  
 5 a transcription termination and polyadenylation signal which functions in plant cells.

A particularly preferred embodiment of the coding region as defined above is a coding region comprising a nucleotide sequence which encodes a protein  
 10 comprising the amino acid of SEQ ID 2, particularly the nucleotide sequence of SEQ ID No 1, or a part of such a nucleotide sequence capable of being translated into a functional sucrose synthase. The coding region encoding the sucrose synthase activity may be derived from other plant species or other species (as indicated elsewhere in this application) and may be, *e.g.*, from  
 15 potato (cDNA sequence is available from Genbank library, accession number M18745).

A particularly preferred embodiment of the coding region as defined above is a coding region comprising the complement of a nucleotide sequence  
 20 ("antisense") encoding a polypeptide encoded by the nucleotide sequence of SEQ ID No 1 from the nucleotide at position 2208 to the nucleotide at position 2598, particularly the coding region comprises the nucleotide sequence of SEQ ID No 1 from the nucleotide at position 2208 to the nucleotide at position 2598.

25 As used herein, a "coding region refers" to a DNA sequence which is capable of being transcribed into a biologically active RNA. Biologically active RNA includes RNA which is capable of inducing a biological effect in the target cell, such as antisense or sense RNA capable of triggering post transcriptional gene silencing, ribozymes etc.

30

Biologically active RNA also includes RNA that is capable of being translated into a polypeptide or protein.

Having read these preferred embodiments, the person skilled in the art will  
 5 immediately realize that functional equivalents of the mentioned coding regions may be used to similar effect.

Functional equivalents of a coding region capable of being transcribed into RNA that is capable of reducing the expression of an endogenous sucrose  
 10 synthase gene include e.g. smaller antisense fragments of the mentioned nucleotide sequences.

The length of the antisense nucleotide sequence may vary from about 10 nucleotides (nt) or 19 nt, up to a length equaling the length (in nucleotides) of  
 15 the target nucleic acid (here a sucrose synthase gene). Preferably the total length of the antisense nucleotide sequence is at least 10 nt, preferably 15 nt or 19 nt, particularly at least about 50 nt, more particularly at least about 100 nt, especially at least about 150 nt, more especially at least about 200 nt, quite especially at least about 500 nt. It is expected that there is no upper limit to the  
 20 total length of the antisense nucleotide sequence, other than the total length of the target nucleic acid. However for practical reason (such as *e.g.* stability of the chimeric genes) it is expected that the length of the antisense nucleotide sequence should not exceed 5000 nt, particularly should not exceed 2500 nt and could be limited to about 1000 nt.

25 It will be appreciated that the longer the total length of the antisense nucleotide sequence is, the less stringent are the requirements for sequence identity between the total antisense nucleotide sequence and the complement of the corresponding sequence in the target sucrose synthase gene. Preferably, the  
 30 total antisense nucleotide sequence should have a sequence identity of at least about 75% with the complement corresponding target sequence, particularly at

least about 80 %, more particularly at least about 85%, quite particularly about 90%, especially about 95%, more especially about 100%, quite especially be identical to complement of the corresponding part of the target nucleic acid.

However, it is preferred that the antisense nucleotide sequence always

- 5 includes a sequence of about 10 consecutive nucleotides, particularly about 19 or 20 nt, more particularly about 50 nt, especially about 100 nt, quite especially about 150 nt with 100% sequence identity to the complement of the corresponding part of the target nucleic acid. Preferably, for calculating the sequence identity and designing the corresponding antisense sequence, the
- 10 number of gaps should be minimized, particularly for the shorter antisense sequences.

Particularly preferred are antisense nucleotide sequences having a sequence identity of at least about 75%, preferably at least about 80%; particularly at

- 15 least about 85%; quite particularly about 90%, especially about 95% with the complement of the corresponding part of the nucleotide sequence of SEQ ID No 1.

For the purpose of this invention, the "sequence identity" of two related

- 20 nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, *i.e.* a position in an alignment where a residue is present in one sequence but not in the other is regarded as a position with non-identical residues. The
- 25 alignment of the two sequences is performed by the Needleman and Wunsch algorithm (Needleman and Wunsch 1970). Computer-assisted sequence alignment, can be conveniently performed using standard software program such as GAP which is part of the Wisconsin Package Version 10.1 (Genetics Computer Group, Madison, Wisconsin, USA) using the default scoring matrix
- 30 with a gap creation penalty of 50 and a gap extension penalty of 3.

It is also understood that sense fragments may be used for reducing the expression of an endogenous sucrose synthase gene, and the same embodiments of length and sequence homology as herein described for antisense molecules, apply *mutatis mutandis* to the sense molecules.

5

Particularly suited for reducing the expression of an endogenous sucrose synthase genes are DNA regions, preferably under the control of a plant-expressible promoter, which when transcribed result in so-called double stranded RNA molecules, comprising both sense and antisense sequences which are capable of forming a double stranded RNA molecule as described in WO 99/53050 (herein entirely incorporated by reference). In this particular case, a chimeric gene may thus be introduced into a plant cell comprising a plant expressible promoter operably linked to a DNA region, whereby that DNA region comprises a part of coding region comprising at least 10 or 19 consecutive nucleotides from the coding region of a nucleic acid encoding a sucrose synthase protein, such as but not limited to, a sucrose synthase protein with the amino acid sequence of SEQ ID No 1 (the so-called sense part) as well as a DNA sequence which comprises at least the complementary DNA sequence of at least 10 or 19 nucleotides of the sense part, but which may be completely complementary to the sense part (the so-called antisense part). The chimeric gene may comprise additional regions, such as a transcription termination and polyadenylation region functional in plants. When transcribed an RNA can be produced which may form a double stranded RNA stem between the complementary parts of the sense and antisense region. A spacer region may be present between the sense and antisense nucleotide sequence. The chimeric gene may further comprise an intron sequence, preferably located in the spacer region.

Functional equivalents of coding regions capable of being transcribed into RNA that can be translated into an active sucrose synthase comprise mutant or

30

allelic forms derived from sucrose synthase genes, particularly from sucrose synthase gene with active expression in fiber initials.

Methods to derive mutants e.g. of a sucrose synthase gene, particularly of sucrose synthase genes encoding a protein as represented in SEQ ID 2, quite particularly of sucrose synthase gene comprising the nucleotide sequence of SEQ ID 1, such a site-specific mutagenesis methods are well known in the art, as well as assays to identify active sucrose synthase enzymes encoded by the mutant sequences.

Allelic forms of the nucleotide sequences encoding sucrose synthase may be identified by hybridization of libraries, under stringent conditions, such as cDNA or genomic libraries of a different varieties or plant lines, particularly cotton varieties and plant lines. Nucleotide sequences which hybridize under stringent conditions to nucleotide sequences encoding the amino acid sequence of SEQ ID 2 or to the nucleotide sequence of SEQ ID 1, or a sufficiently large part thereof (preferably about 25 contiguous nucleotides, particularly at least about 50 contiguous nucleotides, more particularly at least about 100 contiguous nucleotides) and which encode a functional protein with sucrose synthase activity are functional equivalents of the above mentioned preferred coding regions. Such nucleotides may also be identified and isolated using e.g. polymerase chain reaction amplification using an appropriate pair of oligonucleotides having at least about 25 contiguous nucleotides, particularly at least about 50 contiguous nucleotides, more particularly at least about 100 contiguous nucleotides of the nucleotide of SEQ ID No 1.

"Stringent hybridization conditions" as used herein mean that hybridization will generally occur if there is at least 95% and preferably at least 97% sequence identity between the probe and the target sequence. Examples of stringent hybridization conditions are overnight incubation in a solution comprising 50% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium

phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 µg/ml denatured, sheared carrier DNA such as salmon sperm DNA, followed by washing the hybridization support in 0.1 x SSC at approximately 65 °C. Other hybridization and wash conditions are well known and are exemplified in

- 5 Sambrook et al, Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly chapter 11.

The following are accession numbers of nucleotide sequences in the Genbank library which are plant sucrose synthase genes, parts thereof or nucleotide

- 10 sequences having sequence similarity to sucrose synthase genes which could be used according to the methods herein described: BM094593 (*Glycine max*); BM093753 (*Glycine max*); BM093158 (*Glycine max*); BM092695 (*Glycine max*); BM092443 (*Glycine max*); BM092322 (*Glycine max*); BM085310 (*Glycine max*); BM085020 (*Glycine max*); AY059416 (*Zea mays*); AF273253
- 15 (*Beta vulgaris*); L39940 (*Oryza sativa*); AJ316590 (*Nostoc punctiforme*); AJ316589 (*Nostoc punctiforme*); AJ316596 (*Anabaena* sp.); AJ316595(*Anabaena* sp.); AJ316584 (*Anabaena* sp.); BM005654 (*Crocus sativus*); BI973032 (*Glycine max*); BI971794 (*Glycine max*); AF367450 (*Prunus persica*); BI945506 (*Glycine max*); BI944973 (*Glycine max*); AF420224 (*Carica papaya*);
- 20 BI788449 (*Glycine max*); BI788359 (*Glycine max*); BI787127 (*Glycine max*); BI787033(*Glycine max*); BI787000 (*Glycine max*); BI786823(*Glycine max*); BI784933 (*Glycine max*); BI784627 (*Glycine max*); BI700214 (*Glycine max*); BI699934 (*Glycine max*); BI699923 (*Glycine max*); BI699585(*Glycine max*); BI543240 (Sugar beet); BI498340 (*Glycine max*); BI471463 (*Glycine max*);
- 25 BI427241(*Glycine max*); BI427174 (*Glycine max*); BI427022 (*Glycine max*); BI426915 (*Glycine max*); AF393809 (*Apium graveolens*); BI321173 (*Glycine max*); BI320832 (*Glycine max*); BI316894 (*Glycine max*); BI316826 (*Glycine max*); BI316405 (*Glycine max*); BI315949 (*Glycine max*); BI203222 (*Lycopersicon esculentum*); BI176503 (*Solanum tuberosum*); BG273882
- 30 (Grape berries); AY034958 (*Arabidopsis thaliana*); AF378187(*Oryza sativa*); BG790580 (*Glycine max*); BG790079 (*Glycine max*); BG726150 (*Glycine max*);

- BG654021 (*Glycine max*); BG653916 (*Glycine max*); BG653624 (*Glycine max*);  
 BG652711(*Glycine max*); BG652507 (*Glycine max*); BG649914 (*Glycine max*);  
 BG649831 (*Glycine max*); AJ309093 (*Pinus pinaster*); BG507656 (*Glycine*  
*max*); BG405632 (*Glycine max*); BG405474 (*Glycine max*); BG405204 (*Glycine*  
 5 *max*); BG405017 (*Glycine max*); BG363730 (*Glycine max*); BG362638 (*Glycine*  
*max*); BG359764 (*Glycine max*); BG359452 (*Glycine max*); BG359307 (*Glycine*  
*max*); AJ311496 (*Pisum sativum*); BG239317 (*Glycine max*); BG237287  
 (*Glycine max*); BG157592 (*Glycine max*); BG155900 (*Glycine max*); BG047402  
 (*Glycine max*); BG046717 (*Glycine max*); BG046686 (*Glycine max*); BG046043  
 10 (*Glycine max*); BG043243 (*Glycine max*); BG042159 (*Glycine max*); BG041814  
 (*Glycine max*); AB045710 (*Pyrus pyrifolia*); BF597682 (*Glycine max*);  
 BF597330 (*Glycine max*); BF597258 (*Glycine max*); BF595837 (*Glycine max*);  
 BF595646 (*Glycine max*); BF425609 (*Glycine max*); BF423725 (*Glycine max*);  
 BF324657 (*Glycine max*); BF154308 (*Solanum tuberosum*); BF154187  
 15 (*Solanum tuberosum*); BF154037 (*Solanum tuberosum*); BF153500 (*Solanum*  
*tuberosum*); BF153341 (*Solanum tuberosum*); BF153335 (*Solanum*  
*tuberosum*); BF153307 (*Solanum tuberosum*); BF097021 (*Lycopersicon*  
*esculentum*); BF070782 (*Glycine max*); BF070666 (*Glycine max*); BF070277  
 (*Glycine max*); BF070188 (*Glycine max*); BF068815 (*Glycine max*); BF071174  
 20 (*Glycine max*); BF067110 (*Glycine max*); BF009756 (*Glycine max*); BE806119  
 (*Glycine max*); BE805996 (*Glycine max*); BE805178 (*Glycine max*); BE800976  
 (*Glycine max*); BE800941(*Glycine max*); BE800227 (*Glycine max*); BE611805  
 (*Glycine max*); BE611493 (*Glycine max*); BE610447 (*Glycine max*); BE610325  
 (*Glycine max*); BE609887(*Glycine max*); BE609881 (*Glycine max*); BE607382  
 25 (*Oryza sativa*); BE607326 (*Oryza sativa*); BE607323 (*Oryza sativa*); BE556484  
 (*Glycine max*); BE556306 (*Glycine max*); BE555319 (*Glycine max*); AJ001117  
 (*Triticum aestivum*); AJ292758 (*Anabaena variabilis*); BE474178 (*Glycine max*);  
 BE440931 (*Glycine max*); BE440323 (*Glycine max*); BE347277 (*Glycine max*);  
 AF263384 (*Saccharum officinarum*); BE330345 (*Glycine max*); BE211050  
 30 (*Glycine max*); BE209707 (*Glycine max*); AW561929 (*Gossypium hirsutum*);  
 BE040389 (*Oryza sativa*); BE040121 (*Oryza sativa*); BE039563 (*Arabidopsis*

- thaliana*); BE034387 (*Mesembryanthemum crystallinum*); BE033387 (*Mesembryanthemum crystallinum*); BE023957 (*Glycine max*); BE023630 (*Glycine max*); BE022760 (*Glycine max*); BE021167 (*Glycine max*); BE020760 (*Glycine max*); BE020591 (*Glycine max*); BE020550 (*Glycine max*); AW990923  
 5 (*Euphorbia esula*); AW832022 (*Glycine max*); AW760552 (*Glycine max*); AW756812 (*Glycine max*); AW756072 (*Glycine max*); AW756065 (*Glycine max*); AW734901 (*Glycine max*); AW707163 (*Glycine max*); AW706660 (*Glycine max*); AW706520 (*Glycine max*); AW706203 (*Glycine max*); AW705595 (*Glycine max*); AW704619 (*Glycine max*); AB025778 (*Citrus unshiu*); AB021745 (*Citrus unshiu*); AW666333 (*Glycine max*); AW666277 (*Glycine max*); AW666250 (*Glycine max*); AW620859 (*Glycine max*); AW598476 (*Glycine max*); AW598473 (*Glycine max*); AW597690 (*Glycine max*); AW597373 (*Glycine max*); AW597332 (*Glycine max*); AW596993 (*Glycine max*); AW570615 (*Glycine max*); AW570577 (*Glycine max*);  
 10 AW570566 (*Glycine max*); AW570513 (*Glycine max*); AW569822 (*Glycine max*); AW568526 (*Glycine max*); AW568333 (*Glycine max*); AW509231 (*Glycine max*); AW472408 (*Glycine max*); AW459606 (*Glycine max*); AW458318 (*Glycine max*); X82504 (*C. rubrum*); AW432731 (*Glycine max*); AW432392 (*Glycine max*); AW432192 (*Glycine max*); AW397142 (*Glycine max*); AW397071 (*Glycine max*); AW309503 (*Glycine max*); AW307502 (*Glycine max*); AW307391 (*Glycine max*); AW307001 (*Glycine max*); AW306834 (*Glycine max*); AB018561 (*Citrullus lanatus*); AB029401 (*Citrus unshiu*); AB022092 (*Citrus unshiu*); AB022091 (*Citrus unshiu*); AW279073 (*Glycine max*); AW279053 (*Glycine max*); AW278487 (*Glycine max*); AJ388994  
 15 (*Medicago truncatula*); AJ388888 (*Medicago truncatula*); AW234887 (*Glycine max*); AJ238219 (*Triticum aestivum*); AJ238218 (*Triticum aestivum*); AJ238217 (*Triticum speltoides*); AW201670 (*Glycine max*); AW185801 (*Glycine max*); AW185627 (*Glycine max*); AJ249624 (*Triticum aestivum*); AJ249623 (*Triticum aestivum*); AW164630 (*Glycine max*); AW164393 (*Glycine max*); AW133248  
 20 (*Glycine max*); AW101578 (*Glycine max*); AW100191 (*Glycine max*); AW100069 (*Glycine max*); AW099557 (*Glycine max*); X96938 (*T. gesneriana*);  
 25  
 30



- X96939 (*T. gesneriana*); AW035186 (*Lycopersicon esculentum*); AW033439 (*Lycopersicon esculentum*); AW032339 (*Lycopersicon esculentum*); AJ132002 (*Craterostigma plantagineum*); AJ132001 (*Craterostigma plantagineum*); AJ132000 (*Craterostigma plantagineum*); AJ131999 (*Craterostigma plantagineum*);
- 5 AI973811 (*Glycine max*); AI973710 (*Glycine max*); AI973540 (*Glycine max*); AI967739 (*Lotus japonicus*); AI965972 (*Glycine max*); AI960742 (*Glycine max*); AI960703 (*Glycine max*); AI930917 (*Glycine max*); AI900130 (*Glycine max*); AI900087 (*Glycine max*); AI855470 (*Glycine max*); AA080634 (*Saccharum sp.*); AA080610 (*Saccharum sp.*); AA269294 (*Saccharum sp.*);
- 10 AA080580 (*Saccharum sp.*); AI736370 (*Glycine max*); AI731292 (*Gossypium hirsutum*); AI731115 (*Gossypium hirsutum*); AI729201 (*Gossypium hirsutum*); AI728436 (*Gossypium hirsutum*); AI727966 (*Gossypium hirsutum*); AI726092 (*Gossypium hirsutum*); U73588 (*Gossypium hirsutum*); U73587 (*Gossypium hirsutum*); AJ012080 (*Pisum sativum*); AJ131964 (*Medicago truncatula*);
- 15 AJ131943 (*Medicago truncatula*); AJ133726 (*Lotus japonicus*); Y16091 (*Daucus carota*); Y16090 (*Daucus carota*); AJ011319 (*Lycopersicon esculentum*); AI496671 (*Glycine max*); AI496540 (*Glycine max*); AI496532 (*Glycine max*); AI495774 (*Glycine max*); AI495135 (*Glycine max*); AI495023 (*Glycine max*); AI494833 (*Glycine max*); AJ011534 (*Lycopersicon esculentum*);
- 20 Y15802 (*Hordeum vulgare*); AI461126 (*Glycine max*); AI460757 (*Glycine max*); AI460629 (*Glycine max*); AI444096 (*Glycine max*); AI444083 (*Glycine max*); AI444054 (*Glycine max*); AI443620 (*Glycine max*); AI443476 (*Glycine max*); AI443231 (*Glycine max*); AI442789 (*Glycine max*); AI442411 (*Glycine max*); AI441989 (*Glycine max*); AI441004 (*Glycine max*); AI437923 (*Glycine max*);
- 25 AI437907 (*Glycine max*); AI437840 (*Glycine max*); AJ010639 (*Anabaena sp.*); AJ011535 (*Lycopersicon esculentum*); D10266 (*Vigna radiata*); L03366 (*Oryza sativa*); AF030231 (*Glycine max*); M97551 (*Vicia faba*); AJ000153 (*Triticum aestivum*); AF079523 (*Musa acuminata*); AF079851 (*Pisum sativum*); AJ001071 (*Pisum sativum*); AF049487 (*Medicago sativa*); AF054446
- 30 (*Mesembryanthemum crystallinum*); AA753339 (*Oryza sativa*); AA752298 (*Oryza sativa*); AA752293 (*Oryza sativa*); AA753445 (*Oryza sativa*); AA753437

(*Oryza sativa*); AA753297 (*Oryza sativa*); AA752123 (*Oryza sativa*); AA751990 (*Oryza sativa*); AA750692 (*Oryza sativa*); AA750079 (*Oryza sativa*); AA749692 (*Oryza sativa*); AA749554 (*Oryza sativa*); AA720478 (*Mesembryanthemum crystallinum*); AA661050 (*Medicago truncatula*); AA661041 (*Medicago truncatula*); AA660686 (*Medicago truncatula*); D88412 (Cotton); D10418 (Rice); D21308 (Rice); D29733 (Rice); X81974 (*B. vulgaris*); X92378 (*A. glutinosa*); Z56278 (*V. faba*); Z48640 (*V. faba*); X98598 (*P. sativum*); T25261 (*Zea mays*); T23326 (*Zea mays*); T14713 (*Zea mays*); T14662 (*Zea mays*); T14661 (*Zea mays*); X75332 (*D. carota*); X02382 (*Zea mays*); X02400 (*Zea mays*); X70990 (*A. thaliana*); X60987 (*A. thaliana*); X69773 (*V. faba*); X73477 (*S. tuberosum*); Z11532 (*S. officinarum*); Z15028 (*O. sativa*); X64770 (*O. sativa*); X59046 (*O. sativa*); X66728 (*H. vulgare*); X65871 (*H. vulgare*); X69931 (*H. vulgare*); A27685 (*O. sativa*); W21612 (*Zea mays*); U24088 (*Solanum tuberosum*); U24087 (*Solanum tuberosum*); X73221 (*H. vulgare*); L32898 (*Zea mays*); F13913 (*Arabidopsis thaliana*); F13912 (*Arabidopsis thaliana*); U21129 (*Solanum tuberosum*); M26672 (*Triticum aestivum*); M26671 (*Triticum aestivum*); L19762 (*Lycopersicon esculentum*); M18745 (Potato); L33244 (*Zea mays*); L22296 (*Zea mays*); and Z17959 (*Arabidopsis thaliana*). These sequences are incorporated by reference.

20

In a second aspect, the present invention provides a method of altering the production of fiber, fruit or seeds in a plant, the method comprising causing under- or overexpression of an isoform of SuSy involved in fruit/seed production in the plant.

25

Preferably, for underexpression, the method involves providing a genetic construct that targets the 3' end of the SuSy gene when transformed in a plant. The construct can be a co-suppression antisense or combined sense/antisense (inverted repeat) SuSy construct.

30

In a third aspect, the present invention provides a plant having an altered ability to produce fiber, fruit or seeds produced by the method according to the second aspect of the present invention.

- 5 In a fourth aspect, the present invention provides a genetic construct targeting the 3' end of the SuSy gene which, when expressed in a plant, reduces the expression of an isoform of SuSy involved in fruit/seed production.

- 10 Preferred embodiments of the chimeric genes, particularly of the coding regions used for these methods are as described elsewhere in this specification.

- 15 Preferably, the construct is selected from the constructs shown in Figure 5, a combination of parts of both constructs to generate an inverted repeat gene suppression construct, with or without a tissue specific promoter region.

- One preferred method of developing constructs is by using the "pPLEX" technology involving subclover stunt virus promoters and terminators described in AU 689311.

- 20 In a fifth aspect, the present invention provides use of the construct according to the fourth aspect of the present invention to produce a plant having reduced ability to produce fiber, fruit or seeds.

The present invention is suitable for the following applications:

- 25 A) Use of SuSy to improve fiber yield

- SuSy may be used to improve fiber synthesis by increasing SuSy expression or activity in such plant cells, e.g., a fiber specific or fiber enhanced promoter (such as but not limited to a cotton expansin promoter, or the promoter of the SuSy gene having the nucleotide sequence of SEQ ID No 1),  
30 or a constitutive promoter (such as CaMV 35S) can be used to express the SuSy gene in cotton cells.

- Cotton fiber yield can be improved by reducing or suppressing cotton fuzz fiber. Fuzz fiber specific or enhanced promoter or constitutive promoter to reduce sucrose synthase selectively in cotton fuzz fiber cells. Reduced expression can be achieved by gene silencing technologies (antisense, co-suppression, dominant negative etc). Dominant negative mutant alleles of sucrose synthase genes may be obtained, e.g., through mutation (insertion, substitution, or deletion of the phosphorylation site(s) of the sucrose synthase protein coding region)
- 5
- 10        - Cotton fiber yield can be improved by modifying fuzz fibers into lint fibers, e.g., by expression of SuSy coding region or a functional equivalent thereof by fiber specific or fiber enhanced promoter, primary or secondary cell wall promoter or a constitutive promoter.
- 15    B) Use of SuSy gene to improve fiber quality
- Fiber length is an example of fiber quality. Expression of SuSy by a fiber specific or fiber enhanced promoter, primary cell wall promoter or a constitutive promoter.
- 20    C) Use of SuSy to improve fiber properties (e.g. fiber strength, length and number)
- Fiber strength is an example of a fiber property. Fiber strength is significantly affected by the cellulose content in the secondary cell wall of fiber cells. This may be achieved by expression of SuSy by a fiber specific or fiber enhanced promoter, secondary cell wall promoter or a constitutive promoter.
- 25
- D) Overexpression of SuSy, specifically in seeds, to increase sucrose utilization in seeds for increased seed size and storage product content.
- 30    E) Overexpression in the maternal tissue of a fruit (such as cotton fiber, horticultural fruit) for increased carbohydrate or fiber content.

F) Suppression of SuSy in seed with over expression of SuSy in fruit/fiber for increased fiber/fruit yield.

- 5 Preferred plant-expressible promoters include the fiber specific and/or secondary cell wall specific promoters which can be isolated according to the teaching of WO 98/18949, WO98/00549 or US5932713.

- 10 The correlation of sucrose synthase activity in the ovule epidermis of cotton with fiber length also allows the use of sucrose synthase proteins (or antibodies or aptamers recognizing the same) or sucrose synthase coding regions as a diagnostic tool in cotton breeding. These may be used to identify cotton lines or varieties, including wild sources, which have enhanced sucrose synthase activity and increased potential to form longer fibers, particularly when crossed
- 15 in a breeding program with other cotton lines and/or varieties having good fiber characteristics. Nucleic acids derived from sucrose synthase coding regions may be used to determine the amount of sucrose synthase RNA in the ovule epidermis. In addition, polymorphisms, including single nucleotide polymorphisms between sucrose synthase genes may be used to identify lines
- 20 with superior sucrose synthase alleles.

- 25 Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

- 30 The methods and means of the invention are particularly suited for use in cotton plants, (both *Gossypium hirsutum* and *Gossypium barbadense*) particularly for Coker 312, Coker310, Coker 5Acala SJ-5, GSC25110, FiberMax 819, Siokra 1-3, T25, GSA75, Acala SJ2, Acala SJ4, Acala SJ5, Acala SJ-C1,

Acala B1644, Acala B1654-26, Acala B1654-43, Acala B3991, Acala GC356, Acala GC510, Acala GAM1, Acala C1, Acala Royale, Acala Maxxa, Acala Prema, Acala B638, Acala B1810, Acala B2724, Acala B4894, Acala B5002, non Acala "picker" Siokra, "stripper" variety FC2017, Coker 315, STONEVILLE 506, STONEVILLE 825, DP50, DP61, DP90, DP77, DES119, McN235, HBX87, HBX191, HBX107, FC 3027, CHEMBRED A1, CHEMBRED A2, CHEMBRED A3, CHEMBRED A4, CHEMBRED B1, CHEMBRED B2, CHEMBRED B3, CHEMBRED C1, CHEMBRED C2, CHEMBRED C3, CHEMBRED C4, PAYMASTER 145, HS26, HS46, SICALA, PIMA S6 and ORO BLANCO PIMA.

10

Any description of prior art documents herein is not an admission that the documents form part of the common general knowledge of the relevant art.

In order that the present invention may be more clearly understood, preferred forms will be described with reference to the following examples and drawings.

15

#### Modes for Carrying Out the Invention

##### DEFINITIONS

##### **General molecular biology**

Unless otherwise indicated, the recombinant DNA techniques utilized in the present invention are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984); J. Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989); T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991); D.M. Glover and B.D. Hames (editors), DNA Cloning: A Practical Approach, Volumes 1-4, IRL Press (1995 and 1996); and F.M. Ausubel et al. (Editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present). These publications are incorporated herein by reference.

20  
25  
30

Analysis for presence of the transgene by Southern blotting was carried out as described in Sambrook et al. (1989) and immunolocalization of the SuSy protein was carried out on tissue sections as described in Ruan and Chourey (1998).

### Gene/DNA isolation

The DNA encoding a protein may be obtained from any cDNA library prepared from tissue or organisms believed to express the gene mRNA and to express it at a detectable level. The gene sequences can also be obtained from a genomic library or genomic DNA.

Libraries are screened with probes or analytical tools designed to identify the gene of interest or the protein encoded by it. For cDNA expression libraries, suitable probes include monoclonal or polyclonal antibodies that recognize and specifically bind the protein; oligonucleotides of about 20-80 bases in length that encode known or suspected portions of cDNA from the same or different species; and/or complementary or homologous cDNAs or fragments thereof that encode the same or a hybridizing gene. Appropriate probes for screening genomic DNA libraries include, but are not limited to, oligonucleotides; cDNAs or fragments thereof that encode the same or hybridizing DNA including expressed sequence tags and the like; and/or homologous genomic DNAs or fragments thereof. Screening the cDNA or genomic library with the selected probe may be conducted using standard procedures as described in chapters 10-12 of Sambrook *et al.*

An alternative means to isolate a gene encoding is to use polymerase chain reaction (PCR) methodology as described in section 14 of Sambrook *et al.* This method requires the use of oligonucleotide probes that will hybridize to the gene.

The oligonucleotide sequences selected as probes should be of sufficient length and sufficiently unambiguous that false positives are minimized. The actual nucleotide sequence(s) is usually based on conserved or highly homologous nucleotide sequences or regions of the gene. The

5 oligonucleotides may be degenerate at one or more positions. The use of degenerate oligonucleotides may be of particular importance where a library is screened from a species in which preferential codon usage in that species is known. The oligonucleotide must be labeled such that it can be detected upon hybridization to DNA in the library being screened. The preferred method of  
10 labeling is to use  $^{32}\text{P}$ -labelled ATP with polynucleotide kinase, as is well known in the art, to radiolabel the oligonucleotide. However, other methods may be used to label the oligonucleotide, including, but not limited to, biotinylation or enzyme labeling.

15 Nucleic acid having all the protein coding sequence is obtained by screening selected cDNA or genomic libraries, and if necessary, using conventional primer extension procedures as described in section 7.79 of Sambrook *et al.*, to detect precursors and processing intermediates of mRNA that may not have been reverse-transcribed into cDNA.

20 Another alternative method for obtaining the gene of interest is to chemically synthesize it using one of the methods described in Fingels *et al.* (*Agnew Chem. Int. Ed. Engl.* 28: 716-734, 1989). These methods include triester, phosphite, phosphoramidite and H-Phosphonate methods, PCR and other  
25 autoprimer methods, and oligonucleotide syntheses on solid supports. These methods may be used if the entire nucleic acid sequence of the gene is known, or the sequence of the nucleic acid complementary to the coding strand is available, or alternatively, if the target amino acid sequence is known, one may infer potential nucleic acid sequences using known and preferred coding  
30 residues for each amino acid residue.



### Cotton Transformation

Cotton was transformed using an *Agrobacterium*-mediated transformation technique as described in F. Murray et al. 1999 (*Molec. Breeding* 5: 219-232).

Transformed plants were grown in a glasshouse under natural illumination at  
5 day/night temperatures of approximately 30<sup>0</sup> C / 22<sup>0</sup> C.

### Example 1: SuSy-Suppression in cotton.

To provide evidence regarding the role of SuSy in fiber development, the present inventors transformed cotton with co-suppression and antisense SuSy  
10 constructs, targeting the 3' end of the seed SuSy cDNA driven by a constitutive subclover stunt seven virus promoter (see Figure 5 for a schematic representation). The presence of the transgene in 11 transgenic lines so far was confirmed by Southern analysis (see Figure 6). Among them two antisense  
15 lines (294-147, 292-43) and one co-suppression line (295-82) showed dramatic reductions in fiber and seed development with the remaining lines showing various degrees of inhibition of fiber growth.

It was found that:

(a) Immunolocalization analysis on 0-d ovule sections (Figure 2) revealed  
20 that, compared to wild type fiber initials, SuSy protein was reduced to less than 20% wild type levels in fibers of line 294-147 and to undetectable levels in 295-82. Furthermore, the number and size of initiating fibers was reduced by at least 50% in these two lines as compared that in the wild type ovules. Indeed, by 6 days after anthesis (DAA), these transgenic seeds were virtually fiber-less  
25 (see Figure 1). Sucrose synthase activity was determined biochemically. Whereas WT plants show a SuSy activity of 22.5 nmol/min/seed, line 147 showed a SuSy activity of 5.8 nmol/min/seed and line 82 showed no detectable activity. This led to a dramatic reduction of normal fiber initial cells in the ovule epidermis. It is clear from these results that the degree of fiber initiation  
30 depends on the level of SuSy expression in the ovule epidermis.

The impact of SuSy expression on fiber initiation is further demonstrated by scanning electron microscopy analysis of the ovule epidermis of line 147 (see figure 7) revealed that many fiber cells are collapsed and shrunk (see figure 7B). Some fiber cells appeared, but these were much smaller, and were reduced in number when compared to wild type ovule (figure 7A). The shrunk and collapsed phenotype was clearly visible under high magnification (figure 7C).

These results demonstrate that some isoforms of SuSy play a critical role in fiber initiation and elongation. Suppression of an isoform of SuSy can thus be used to prevent or reduce fiber initiation and elongation while overexpression can thus be used to enhance fiber initiation and elongation.

(b) While all the bolls in line 295-82 and 292-43 dropped off prematurely by 10 DAA, some bolls of line 294-147 were retained to maturity. In those mature bolls, most seeds were stunted, shrunken and fiberless. About 15% of the seeds, however, showed 30% of the wild type fiber length (loose fiber as well) and wild type embryo and seed size, most likely due to segregation of the transgene.

This demonstrates that suppression of isoforms of SuSy in the maternal tissue (seed coat/ fiber) alone can inhibit fiber development by ~70% in length, while additional repression of SuSy in the embryo can arrest seed development entirely.

Gene suppression of isoforms of SuSy in the embryo specifically to give a seedless phenotype (in a wide range of horticultural crops such as grape, peach, pear, apple).

(c) Line 292-43 and 295-82 were male-sterile (no pollen) and the seed set can be recovered by pollination with wild-type pollen. This shows SuSy plays a role in male sterility of cotton.

- 5 Gene suppression of SuSy in male floral parts was found to cause sterility. Sucrose synthase activity, the fresh weight of pollen and viability of the pollen were tested on developing anthers 2 days before flowering in different lines. The viability assay was conducted by staining with 2,3,5-triphenyltetrazolium chloride (TTC). The results are summarized in Table 1.

10

Table 1. Suppression of SuSy in anthers leads to male sterility by pollen formation inhibition.

Line	SuSy activity (nmol min <sup>-1</sup> mg <sup>-1</sup> )	Pollen fresh weight (% of WT pollen per anther)	Viable pollen (% of 100 grains)
WT	44.1 ± 1.1	100.0	85.2
147#1	29.5 ± 6.9	30.0	52
147#2	14.7 ± 0.9	0.0	0.0
82	18.2 ± 1.5	0.0	0.0

- A reduction of SuSy activity in anthers by about 33% results in an about 70%  
 15 reduction of total pollen weight, largely due to the reduction in the amount of pollen produced. Of the residual 30%, only half of the pollen was viable. When SuSy was reduced by about 60%, no pollen was formed. Pollen development is thus very sensitive to changes in SuSy activity. Pollen formation can thus be influenced (inhibited or promoted) by suppressing or overexpressing SuSy in  
 20 anthers.

Suppression of SuSy activity in the maternal seed coat tissue in the transgenic cotton plants also led to reduced fuzz length in the SuSy suppressed transgenic plants when compared to a wild type plant (see Figure 9). Fuzz fiber

length was measured from the chalazal end of the mature seeds and the measurement was done by gently stretching the fuzz of seeds, delinted by hand, and measuring the length from the seed coat epidermis to the tip. The brown color visible in the transgenic line is because fuzz is shorter in transgenic lines than in WT, where the brown seed coat is well covered by relatively long fuzz. SuSy activity was also determined. The results can be summarized as follows:

- the wild type plant had a fuzz length of 2.2 mm (and a SuSy activity measurement set at 100%)
- transgenic line 147#2 had a fuzz length of 1.4 mm (and a SuSy activity measurement of 36% of the WT).

From the gene suppression experiments, it will be understood that overexpression of SuSy gene will have potential beneficial effects on fiber, fruit and seed production in plants.

#### **Example 2. Further analysis of progeny of cotton lines 82, 147 and 43 with suppressed SuSy activity.**

To confirm the correlation between the presence of the SuSy suppression transgene and the reduced fiber initiation in cotton, further analysis was conducted on the T1 generation. About 24 T1 seeds were sown from six T0 lines. All germinated seedlings were screened by PCR for presence or absence of the transgenes (nptII; S7 promoter and SuSy suppression transgene). Six PCR positive and one PCR negative segregating individuals from each of the six lines were further analysed on molecular biochemical and cellular level. Line 82 was propagated vegetatively as a reference point. The fiber-less phenotype in the different lines of the T0 generation (indicated by arrows in figure 6A) was still preserved in respectively three and two segregating individuals of the T1 generation for line 147 and 43 respectively (arrows in Figure 6B). The

remaining individuals show various degree of fiber / seed suppression. The vegetatively kept line 82 continues to produce fiberless seeds. These results demonstrated that the fiberless / or seed suppressed phenotype described was indeed due to the presence of SuSy transgene.

5

A positive strong correlation could be found between the degree of sucrose activity in the seed epidermis and the fiber length in segregating T1 generation plants of lines 147, 43 and 101 (see Figure 8). When fiber length was plotted against sucrose synthase activity for the different plants of the different lines as well as the wild type line, a linear regression could be derived ( $Y=2.634X-50$ ;  $R^2=0.98$ ). The linear nature of the correlation up to the wild-type level, indicates that overexpression of the sucrose synthase activity above wild type level will lead to fiber length greater than fiber length in wild type plants.

10

15

A similar strong correlation exists between the reduction in SuSy activity and mature cotton fiber length and dry weight. SuSy activity in seed coat epidermis has been reduced to 28.8%, 42.5% and 53.9% of the wild type level in segregating individuals #1, #2 and #3 of line 43 T1 generation plants respectively. Lint fiber dry weight per boll was 11.1%, 51.6%, and 70.9% respectively.

20

Cellulose content in cotton fiber is also closely correlated to the level of SuSy expression. Cellulose content was analysed by labeling cotton fiber at day 25 (peak of cellulose synthesis *in vivo*) with Calcofluor white, a fluorescent dye specifically binding to cellulose. The fluorescent intensity of the labeled fibers, indicating cellulose content, was significantly reduced in the SuSy-suppressed transgenic lines when compared to fluorescence in the wild type. The degree of fluorescence reduction correlated to the degree of suppression of SuSy activity.

25

### Example 3. Overexpression of sucrose synthase in cotton plants.

The coding sequence of a potato sucrose synthase cDNA (Genbank Accession number M18745) is operably linked to a subterranean clover stunt virus promoter (S7; WO9606932) and a 3' transcription termination and polyadenylation signal functional in plants. This chimeric gene is operably linked to a selectable marker gene and introduced into a T-DNA vector. Cotton plants are transformed using the above-mentioned *Agrobacterium*-mediated transformation technique. Transgenic cotton lines are identified; sucrose synthase activity, fiber length, fuzz fiber length, cellulose content, and dry weight of the lint are analyzed. A positive correlation is found between SuSy activity and increased fiber length cellulose content, and dry weight of the lint.

### SUMMARY OF THE EXAMPLES

The single-cell cotton fibers initiate from ovule epidermis at anthesis, elongate to 2.5 ~ 3.0 cm in about 16 days and then synthesize massive amounts of cellulose. Thus, cotton fiber is an excellent system for the study of cell differentiation, elongation and cellulose synthesis in higher plants with significant industry implications for improving fiber yield and quality.

To provide definitive evidence regarding the role of SuSy in fiber and seed development, the present inventors transformed cotton with co-suppression and antisense SuSy constructs, targeting the 3' end of the seed SuSy cDNA driven by subclover stunt seven virus promoter. The presence of the transgene in nine transgenic lines so far was confirmed by Southern analysis. Among them one antisense line (294-147) and one co-suppression line (295-82) showed dramatic reductions in fiber and seed development with the remaining lines showing various degrees of inhibition of fiber growth. Immunolocalization analysis on 0-d ovule sections revealed that, compared to wild type fiber initials, SuSy protein was reduced to only about 20% of wild-type levels in fibers of line 294-147 and to undetectable levels in 295-82. Furthermore, the number and size of initiating fibers was reduced by at least 50% in these two

lines as compared that in the wild-type ovules. Indeed, by 2 d after anthesis (DAA), these transgenic seeds were virtually fiberless. This is in contrast to a fiber-covered seed phenotype seen in the wild type plants at this stage. While all the bolls in line 295-82 dropped off prematurely by 10 DAA, some bolls of line 294-147 were retained to maturity. In those mature bolls, most seeds were stunted, shrunken and fiberless. About 15% of the seeds, however, showed 30% of the wild type fiber length and wild type embryo and seed size, most likely due to segregation of the transgene. These results demonstrate (a) that SuSy plays a critical role in fiber initiation and elongation, and (b) that suppression of SuSy in the maternal tissue (seed coat/fiber) alone can inhibit fiber development while additional repression of SuSy in the embryo can arrest seed development entirely. The influence of SuSy suppression on the reduction of fiber length has been confirmed by analysis of progeny of the transgenic cotton lines. Further, a linear correlation was found between the level of SuSy activity and the increase in fiber length. The linear nature of this correlation indicates that in wild type cotton, the level of sucrose synthase is limiting.

Overexpression of sucrose synthase at least in maternal ovule tissue increases fiber length in fiber-producing plants.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

#### References:

Amor , Y., Haigler, C.H., Johnson, S., Wainscott, M. and Delmer, D.P. (1995) A membrane associated form of sucrose synthase and its potential role in

synthesis of cellulose and callose in plants. *Proc Nat Acad Science USA* 92: 9353-9357.

- 5 Ruan, Y.-L., Llewellyn, D.J, Furbank, R.T. (2000) Pathway and control of sucrose import into initiating fiber cells. *Australian Journal of Plant Physiology* 27:795-800.

- 10 Ruan, Y.-L. and Chourey, P.S. (1998) A fiberless seed (fls) mutation in cotton is associated with lack of fiber cell initiation in ovule epidermis, alteration in sucrose synthase expression and carbon partitioning in developing seed. *Plant Physiology* 118: 399-406

- 15 Ruan, Y.-L., Chourey, P.S., Delmer, D.P. and Luis, P.G. (1997) The differential expression of sucrose synthase in relation to diverse patterns of carbon partitioning in developing cotton seed. *Plant Physiology* 115: 375-385